inactivating an endogenous heavy-chain locus by virtue of a lesion in the J region. The dependent claims contain limitations which require additional features in the modification to the germline of the transgenic animal. Support for the introduction of the lesion(s) and xenogeneic loci into the germline is found, for example, on page 11, line 25, page 29, line 14, page 73, lines 1-9 and throughout the specification. As is made clear in the specification, the transgenic mice of the invention breed true to the modifications which have been introduced. No new matter has been added and entry of the amendment is respectfully requested.

Restriction Requirement and Formalities

The proposed claims are directed to the subject matter of the elected invention of Group IV. Claims directed to nonelected inventions have been cancelled.

Applicants verify that subject matter of the various claims now pending and previously pending was commonly owned at the time of any inventions covered.

The phrase,"and/or" no longer appears in the claims.

The rejection of the previous claims under 35 U.S.C. § 112, paragraph 2 also criticized the phrase "inability of at least one locus to produce". Although applicants believe that this language was in conformance with the statute, the present claims provide for a lesion which results in the inability of the copy of the locus to rearrange or to produce a functional message encoding an immunoglobulin heavy-chain subunit. This phrasing clearly meets the statutory test as described in In re Moore, 169 USPQ 236, 238 (CCPA 1971) which states that the analysis of

claims under 35 U.S.C. § 112, paragraph 2, "is merely to determine whether the claims do, in fact, set out and circumscribe a particular area with a reasonable degree of precision and particularity." Thus, the requirements of this section do not stipulate that the claim language suggest a mechanism whereby the circumscribed area may be achieved, as the Office appears to suggest. The wording in the amended claims clearly circumscribes a particular area to be claimed, which is all that is required by the statute.

The further objection to "one locus to produce endogenous immunoglobulin heavy and light chains" is believed mooted by the amendment to the claims, as is this rejection as applied to claims 78-81. It will be noted that the claims as amended require that the germline of the animal contain a modified genome. The modification in the genome may, of course, have a multiplicity of aspects, so that, for example, the modification may include not only a lesion in the J region of one copy of the immunoglobulin heavy-chain locus as required by claim 83, but may also include the insertion of an immunoglobulin locus encoding a xenogeneic light chain, for example.

The Rejection Under 35 U.S.C. § 112, First Paragraph

There appear to be several aspects to this rejection. The first aspect relates to scope as to the nature of the nonprimate mammal employed. The Office states that the claims must be limited to mice with the relevant modifications because the nucleotide sequences of immunoglobulin genes of other nonhuman mammals are assertedly not available in the art.

Applicants certainly agree with the Examiner that the appropriate design of the gene inactivation vectors

required to create the claimed lesion in the J region demand knowledge of the nucleotide sequence in the region to be targeted. However, the nucleotide sequences of the targeting vectors are not claimed and do not constitute the invention. The invention is directed to animals whose germline contains a gene modified according to the directions provided in the specification. These directions presuppose a knowledge of the nucleotide sequence of the targeted region. The claimed invention thus presupposes that the relevant nucleotide sequence for the mammal whose immunoglobulin locus is to be targeted is known or can readily be obtained.

This is certainly the case. A routine machine-based search reveals a substantial body of literature relating to the immunoglobulin genes of nonhuman animals, and representative abstracts printed from Medline are attached. For example, immunoglobulin variable genes from rat hybridomas were isolated (Kutemeier *et al.* Hybridoma (1992) 11:23-32), sequences from rat immunoglobulin lambda chain variable genes were presented (Aguilar *et al.* Immunogenetics (1992) 37:39-48), and a cDNA clone encoding a porcine immunoglobulin mu chain and the complete sequence of the clone is presented (Bosch *et al.* Dev Comp Immunology (1992) 16:329-337). Additionally, immunoglobulin genes have been genetically characterized through comparative gene mapping in cattle (Tobin-Janzen Immunogenetics (1992) 36:157-165), a bovine immunoglobulin gamma heavy-chain gene was assigned to a particular chromosome by *in situ* hybridization (Gu *et al.* Hereditas (1992) 117:237-240), and nucleotide sequences and expression of cDNAs for bovine antitestosterone monoclonal IgG1 antibody were reported (Jackson *et al.* Mol Immunology (1992) 29:667-76). Sheep

immunoglobulin light-chain loci were analyzed through cDNA probes (Foley *et al.* Animal Genetics (1992) 23:31-42). A horse Ig lambda gene was characterized (Home *et al.* J Immunology (1992) 149:3927-3936). Even nonmammalian species have been addressed: cDNA clones encoding chicken Ig H chain isotopes were isolated by Mansikka J Immunology (1992) 149:855-61).

Additionally, the literature reports several comparative studies of animal immunoglobulin genes. For example, heavy-chain joining genes in the rat were compared with those from mouse and humans (Lang, *et al.* Gene (1991) 102:261-64). The rearrangement of immunoglobulin variable-region genes in chicken B cell development was reported as occurring by a molecular mechanism "highly conserved between mammalian and avian species." (Benatar *et al.* PNAS USA (1992) 89:7615-19). Four novel epsilon-chain mRNAs were isolated and comparative analyses of IgE in rodents and man was reported (Hellman European Journal Immunology (1993) 23:159-67).

Other interspecies studies have reported, for example, the expression of mouse IgA by transgenic mice, pigs and sheep (Lo *et al.* European Journal Immunology (1991) 21:1001-6). Additionally, genes encoding a mouse monoclonal antibody were expressed in transgenic mice, rabbits and pigs (Weidle *et al.* Gene (1991) 98:185-91). Other literature describes the *in vitro* propagation of embryonic stem cells that "may be isolated from animals including, but not limited to, humans and a number of other animal species such as birds (e.g., chickens), mice, sheep, pigs, cattle, goats and fish". See U.S. Patent No. 5,166,065.

This body of literature clearly suggests that any "nonavailability" of the appropriate nucleotide sequences

reflects not a requirement for undue experimentation in order to obtain them, but rather a comparative lack of interest in the nucleotide sequences associated with other mammalian species as compared to the more conveniently studied mice and the understandable anthrocentric interest in human genes. There appears to be no evidence of record that obtaining a nucleotide sequence for the targeted area in any arbitrarily chosen mammalian species would involve undue experimentation. Accordingly, this aspect of the § 112 first paragraph basis for rejection may properly be withdrawn.

The second aspect of the rejection under 35 U.S.C. § 112, first paragraph, relates to the region of the immunoglobulin locus to be targeted. This basis for rejection has in part been obviated by amendment; claim 83 requires a lesion in the J region of the endogenous heavy-chain locus. With respect to claim 84, a lesion in either the J or constant region (or, of course, both) is supported by the specification, for example, on page 16, lines 16-30. Although a lesion in the constant region of the κ or light-chain locus is used for illustration, there is no reason of record to doubt the teachings in the specification that either the J or constant region could be targeted and result in the desired inactivation. Nor is there any reason to doubt that the λ light-chain gene could be inactivated in a manner similar to the κ light-chain gene. Accordingly, the present claims are clearly within the scope taught by the specification. This aspect of the rejection under 35 U.S.C. § 112, first paragraph, may also therefore be withdrawn.

The third and final aspect of this rejection resides in the expressed doubt that the genetically modified mouse having "the J region or C_κ region knocked out and a

xenogeneic immunoglobulin gene inserted would produce xenogeneic antibodies in response to an antigenic challenge." The basis for these doubts is said to be the possibility that endogenous variable regions would somehow be inserted into the antibodies produced. Even if this were true, it would not justify the conclusion that fully xenogeneic antibodies could not be produced. At best, it might lead to the conclusion that some of the B cells might produce chimeric antibodies; it does not lead to the conclusion that all B cells would do so. Since it is well known that individual B cells can be immortalized to create hybridomas secreting monoclonal antibodies, production and recovery of fully human antibodies would clearly be possible so long as some B cells contained the appropriately rearranged genes.

Indeed, this is the teaching of the Bruggemann paper cited by the Office (Proc Natl Acad Sci USA (1989) 86:6709-6713). That paper shows that human immunoglobulin minigenes inserted into transgenic mice can rearrange in the lymphocytes of these mice to produce antibodies with human heavy chains. Although for reasons of convenience the "human" mini-IgH locus used by Bruggemann was deliberately chimeric (see page 6710, left-hand column), Bruggemann states clearly, "It should be possible in the future to make a repertoire of fully human antibodies that, unlike chimeric antibodies or antibodies with grafted hypervariable regions, will have no sequences of foreign origin by using mice containing heavy and light chain miniloci." (See page 6712, left-hand column.) It will be noted that what was established to have occurred in the Bruggemann studies was the rearrangement of a single inserted locus which included substantial human regions.

The Office suggests, however, that the heavy-chain variable regions exist on more than one chromosome and that such regions are used in the antigenic response. The Office suggests that since the endogenous variable heavy-chain regions are not knocked out, variable heavy regions from other chromosomes may be used, citing Matsuda *et al.* Nature Genetics (1993) 3:88 in support. However, this article concerns the mapping of the unrearranged human heavy-chain locus on chromosome 14 which contains the J_H segments essential for somatic generation of the V_H gene. The article goes on to state, "Expression of V_H segments on chromosomes 15 and 16 requires interchromosomal recombination which has not been demonstrated in B lymphocytes." Thus, the Matsuda article teaches that what the Office fears might happen has never been shown to occur. In any event, even if it did occur, this would not preclude the formation of fully human antibodies in at least some B cells.

The Office goes on to state that there is "no evidence presented in the specification that without knocking out the endogenous V_H regions the antibodies so produced would be purely xenogeneic." Particularly in view of the teachings of Bruggemann and Matsuda, no such evidence should be necessary. The Office has the burden of establishing a lack of enablement. In re Hogan, 194 USPQ 527, 539 (CCPA 1977). The specification clearly states that human antibodies will be produced by animals which have been modified to contain xenogeneic loci. Absent a valid reason to doubt applicants' statement, it must be accepted at face value. (*In re Marzocchi*, 169 USPQ 367 (CCPA 1967)). *In re Marzocchi* at 369 (supra).

Even when "unpredictability" in a field such as chemistry may create reasonable doubt as to the accuracy of a broad statement supporting enablement, and even when the statement is, on its face, contrary to generally accepted scientific principles, the Court of Customs and Patent Appeals (predecessor to the U.S. Court of Appeals for the Federal Circuit), has clearly articulated that

> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with a contested statement.

The Office also raises the disclosure of Shin. Applicants assume reference is made to the article submitted from EMBO Journal (1991) 10:3641-3645. This article simply indicates that autoantibody-related variable segments are clustered in the heavy-chain locus of the human. Presumably the Office extrapolates from this article to assume that similar regions may occur in the endogenous mouse loci and that these regions would be assembled into antibodies immunospecific to mouse tissue. There is not one iota of evidence on record to support this theory. Even if this theory were accurate, the same problem would also arise in the course of preparing antibodies to a desired antigen in mice *per se*, an exercise that has been successfully performed for decades. Applicants are not aware of any inability of practitioners to prepare antibodies to a desired antigen in mice because the production of autoantibodies postulated by the Examiner got in the way.

There is no reasoning offered to suggest that the situation should be different in the claimed mammals.

Finally, and perhaps most important, the attention of the Office is drawn to the enclosed paper, Green, L.L. et al., Nature Genetics (1994) 7:13-21 which describes the work of Applicants herein and reports the successful preparation and recovery of fully human monoclonal antibodies from mice modified according to the invention and administered the tetanus antigen. See, in particular, the abstract and the left hand column on page 18.

Accordingly, this aspect of the rejection under 35 U.S.C. § 112, first paragraph, may also be withdrawn.

The Rejection Under 35 U.S.C. § 103

The claims as previously pending were rejected as obvious over Bruggemann taken with any of Joyner *et al.*, Thomas *et al.* and Koller *et al.* in further view of Fell. This combination of references, it is believed, is relevant now only to claims 86-88 and claims dependent thereon. As the sole independent claim now presented requires only a lesion in the J region of the endogenous heavy-chain locus as it occurs in the germline of the mammal, it would appear that the Bruggemann reference is not relevant and the Office would have applied only the secondary and tertiary references had the claims been in their present form.

On this basis, the rejection is respectfully traversed.

The claims as amended specifically require that a lesion be present in the J region of the immunoglobulin heavy-chain locus and that the genome be contained in the germline of the transgenic animal. None of the cited references suggests inactivating an entire immunoglobulin

locus by targeting the J region or suggests that such a modification could be carried in the germline.

Thomas describes the inactivation of the HPRT gene in mouse ES cells by homologous recombination. In this rather preliminary study, the authors merely showed that the ES cells themselves contained inactivated genes. No transgenic animals were created. Furthermore, the targeted gene is singularly straightforward and it would have been predictable in advance that the replacement of an endogenous sequence or insertion of an exogenous sequence would inactivate the gene. In any event, this article provides no information as to how to inactivate an immunoglobulin locus, the complexity of which, compared to HPRT, is well understood.

The paper by Koller also describes inactivation of a different gene from that targeted by applicants -- the β_2 microglobulin gene in embryonic stem cells. This paper describes a more complex procedure than that of Thomas since selection must now be provided by a marker. Chimeric mice are obtained using selected ES cells containing inserts in the β microglobulin genes; although the authors express the hope that the chimeric animals can be bred to transmit the inactivated gene to their progeny, the interbreeding experiment was not performed, so it is unclear whether the inactivated gene entered the germline.

The interbreeding experiment was performed in the third paper by Joyner *et al.* and resulted in failure. In the Joyner paper, homologous recombination in murine ES cells was used to inactivate the En-2 gene. This gene was of interest because it is instrumental, apparently, in embryonic development and is expressed in the ES cells *per se*. According to Joyner, ES cells which contained the

inactivated gene successfully produced chimeric males. However, when these males were bred, none of the offspring contained the inactivated gene (see page 155, right-hand column). It therefore appears the inactivated gene was not included in the germline.

Taken together, the three references describing homologous recombination in ES cells merely show that such homologous recombination can be obtained with respect to those genes for which results are disclosed and that the modifications in the genome can be retained in chimeric animals resulting from the implantation of these ES cells. The articles do not demonstrate that the inactivated genes are present in the germline of the chimeric animals or that offspring can be obtained with these modified genes in their germlines. Furthermore, there is no suggestion whatsoever in these references to inactivate the heavy-chain locus at all, and certainly not by targeting the J region.

These deficiencies in the Joyner, Thomas and Koller references are not cured by Fell. The Fell patent is directed to the use of homologous recombination to make chimeric antibodies. Already rearranged genes encoding specific immunoglobulin molecules contained in hybridomas are modified so as to replace the constant region encoded by the endogenous gene with the constant region of another species. The targeted immunoglobulin genes are expressed in the hybridoma cells, are already rearranged, and <u>are not inactivated</u> by this procedure.

Thus, none of the deficiencies of the secondary references is cured. Fell does not verify that the resultant of homologous recombination of any gene can be successfully included in the germline of a transgenic animal. Neither does Fell demonstrate that the

immunoglobulin gene can be inactivated by homologous recombination. And certainly the Fell reference in no way suggests that the J region specifically should be targeted for this purpose.

Thus, claim 83 is clearly unobvious over the art. As the remaining claims contain additional limitations, they too are not suggested by the art. On this basis, the rejection of the pending claims over the art may properly be withdrawn.

Conclusion

The claims have been amended more particularly to focus on the invention. The amended claims make clear that the modified genome must reside in the germline of the transgenic mammal. The claims specifically require also that the J region of the heavy-chain locus be targeted in order to inactivate this locus. None of the cited art suggests this method of inactivating an endogenous immunoglobulin locus. Accordingly, claims 83-103 are in a position for allowance and passage of these claims to issue is respectfully requested.

Respectfully submitted,

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